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Intrinsic Noise Propagation in Two-Step Series Enzymatic Cascades

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Series enzymatic cascades such as MAPK cascades act as signal amplifiers and play a key role in processing information during signal transduction in cells. Cell-to-cell variability or noise, consisting of both intrinsic and extrinsic noise, propagate along with the signal through these cascades and thereby strongly affect the cellular response. Characterization of noise propagation in such cascades can provide insights on the ability of the cells to effectively process information in the presence of noise. Accurate estimation of noise in the cascade is essential for noise propagation studies. Based on the stochastic simulations and global sensitivity analysis, we develop a systematic methodology to characaterize noise propagation in the cascade. Using this method, we show that the noise propagation depends on the total upstream enzyme concentration and the total substrate concentrations.

We prove that the conventional linear stability analysis method applied to the Langevin type stochastic model of the cascade fails to accurately predict the intrinsic noise propagation. This is due to the fact that the effect of nonlinearity on intrinsic noise propagation is ignored in the linear stability analysis method of noise estimation. We prove this by conducting extensive stochastic simulations of the Langevin type model equation and Gillespie simulations of the chemical master equation. Based on the global sensitivity analysis and Gillespie simulations, we show that this conclusion is valid for a wide range of parameters.

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Single-Tryptophan Phosphorescence of Human Serum Albumin Embedded in Sugar Matrices: Protein Dynamics on the Millisecond to Second Timescale

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Long-term stability of amorphous biomolecules is often improved via the inclusion of small-molecule excipients. The effect on slow protein dynamics caused by substitution of a protein's surface water molecules with small sugar molecules is unclear. To explore this question, we have conducted a series of luminescence studies on human serum albumin (HSA) in the dry amorphous solid state. Tryptophan phosphorescence is an ideal approach, as the long-lived triplet state is sensitive to the long timescale molecular motions of the protein in the dry state. The experiments provide evidence that slow protein dynamics are coupled to those of the embedding sugar matrix. HSA also binds small molecular probes which may report a variety of information at a site on the protein's surface. The water-sensitive charge-transfer probe 8-hydroxypyrene 1,3,6-trisulfonate (pyranine) was bound to HSA and its fluorescence spectra were measured to extract information on the amount of the water in the protein's hydration shell. Dry films from sugars doped with both magnesium ion and glycerol were also made to explore the effect of osmolyte and plasticizer. Magnesium sequestered water from the protein's hydration shell and drastically slowed protein dynamics. Glycerol, which is known to have a rigidifying effect on the ps timescale, acts as a plasticizer of protein dynamics on the ms to s timescale.

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Molecular Dynamics Simulations of Cross-Linked Peptides and Enzymes

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Protein engineering typically utilizes amino acid mutations within a protein sequence to enhance activity, stability, or selectivity. We present a new approach to protein engineering by incorporating cross-linkers at predetermined locations in the protein structure, a method we term Site-Directed Cross-Linking (SDXL). SDXL is a promising strategy for studying the structure, function, and dynamics of enzymes. Together, cross-linking and mutation studies offer great insight into the fundamental biophysics of enzymatic systems.

We first applied this method to a set of model helical peptides in order to build a predictive computational model of reaction rates for ring-closure cross-linking reactions. Jacobsen-Stockmeyer theory predicts that entropy is the sole rate-determining factor for ring-closure reaction rates. By computing the difference in conformational entropy between the fully cross-linked peptide and the uncross-linked mono-adduct, we are able to reproduce the trends in experimentally measured cross-linking reaction rates in the set of model helical peptides. These trends highlight the helical structure of the model peptides and demonstrate the potential of SDXL for probing the secondary and tertiary structure of larger, more complex, proteins.

Dihydrofolate reductase (DHFR) is an enzyme that is critical for the biosynthesis of purines. As such it is a common target for antibiotics, cancer therapies, and antimalarial drugs. We have initiated SDXL studies on the e. coli DHFR to investigate whether it is possible to modulate enzyme activity in a rational and controlled way. A single cross-linker was designed and introduced by SDXL between the active-site M20 loop and the distal FG loop that is essential for stabilizing the closed (and active) M20 conformation. We observe how the perturbations introduced by various forms of the cross-linker change the dynamics of the protein, and correlate these structural and dynamic changes with enzyme activity.

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Understanding Alzheimer's: Applying Bioanalytical Techniques for Time-Dependent Structural and Kinetic Studies of the Italian (E22K) Familial Mutation in Aß22-35 Peptide

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Many neurodegenerative diseases, such as Alzheimer's Disease (AD), are perpetuated by the misfolding of proteins and irregular molecular cleaving due to the presence of the detrimental ß -secretase enzyme. The result is beta-amyloid plaque aggregation throughout the hippocampus, ultimately causing the neural death and cognitive dysfunction commonly associated with manifestations of dementia. in order to gain a better understanding of beta-amyloid's mechanisms and kinetics, our research has focused upon the study of the Aß22-35 region of the full Aß1-40 peptide chain because of the strength of the ionic salt bridge and various familial mutations that occur throughout the hair pin region. One such mutation is the Italian (E22K) mutant, distinguished by its substitution of lysine in lieu of glutamic acid at codon 22. Through the implementation of rarely used bioanalytical techniques such as Attenuated Total Reflectance Infrared (ATR-IR) spectroscopy, Ultraviolet Visible (UV-Vis) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy, we observed the peptide's structural changes from monomers to oligomers to beta-sheet intermediates to final beta-sheet aggregations. These techniques both enabled study of the rate of fibril aggregation in time-dependent studies. Our research on the Wild Type (WT Aß22-35) peptide has served as a solid reference of comparison for the mechanisms of E22K mutant. Likewise, earlier studies by Chimon have demonstrated increased toxicity during the peptide's structural changes to beta-sheet intermediates, which we hope to confirm in both the WT and familial mutations. Further studies include Transmission Electron Microscopy (TEM) and Circular Dichroism (CD), which will provide the final confirmation of secondary structure aggregation at our determined critical hours, and toxicity studies that will determine the mutant's intermediates' crucial impact on neurodegeneration.

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A Master Switch Couples Mg^{2+}-Assisted Catalysis to Domain Motion in *B. Stearothermophilus* Tryptophanyl-tRNA Synthetase

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Tryptophanyl-tRNA synthetase (TrpRS) uses conformation-dependent Mg^{2+} activation to couple catalysis of tryptophan activation to specific, functional domain movements. Rate acceleration by Mg^{2+} requires ~ −6.0 kcal/mole in protein•Mg^{2+} interaction energy, none of which arises from the active site. A highly cooperative interaction between Mg^{2+} and four residues from a remote, broadly conserved motif that mediates the shear of domain movement: (i) destabilizes the pre-transition state conformation, thereby (ii) inducing the Mg^{2+} to stabilize the transition state for k_{cat} by ~ −5.0 kcal/mole. Thermofluor studies of differential conformational stability in single mutants to four residues in the motif verify that the conformational changes impact catalysis. Steady-state kinetic analysis of all possible combinations of these four mutations assayed with Mg^{2+} and Mn^{2+} comprises a 5-dimensional multi-mutant thermodynamic cycle. The coupling energies of this cycle are dominated by the 5-way interaction between all four residues of the remote motif and the metal. The coupling energy of this high-order interaction provides 5 kcal/mole in transition state stabilization. This value represents the equilibrium constant for the five components. The thermodynamic differential stability analysis implies that the relevant process is the coupling between the conformational change and the metal activation. Cooperative, long-range conformational effects on the metal therefore convert an inactive Mg^{2+} coordination into one that can stabilize the transition state if, and only if, domain motion occurs. Conformation-dependent Mg^{2+} activation, analogous to the escapement in